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Research Article

Assessment of Mycotoxigenic Fungal Contaminants in Dried Fruits and Nuts in Dhaka City, Bangladesh

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Abstract

Dried fruits and nuts are nutritious but susceptible to fungal contamination and mycotoxin-related health risks. This study investigated the prevalence and diversity of fungal contamination in dried fruits and nuts collected from local markets in Dhaka, Bangladesh. Different varieties of widely consumed dates, raisins, and nuts were collected from markets across Dhaka city for analysis. Fungal contamination was assessed using the direct plating method, with preliminary identification based on morphological and microscopic features, and molecular confirmation using ITS1 and ITS4 primers followed by sequence analysis. The mean fungal colony counts were 84.5 ± 3.20 CFU/g for nuts, 72.8 ± 3.81 CFU/g for raisins, and 58.0 ± 4.24 CFU/g for dates. A total of ten fungal species belonging to four genera—*Aspergillus*, *Penicillium*, *Rhizopus*, and *Fusarium*—were isolated from the samples. *Aspergillus* spp. were the dominant, with *A. niger* and *A. flavus* found in 95.23% and 80.95% of the samples, respectively. Among *Penicillium* species, *P. oxalicum* was the dominant (85.71%). *Rhizopus spp. and Fusarium spp. were detected in 47.6% and 23.8% of samples, respectively.* The findings reveal a high prevalence of potentially toxigenic *Aspergillus* and *Penicillium* in dried fruits and nuts in Bangladesh, underscoring the need for routine monitoring and improved storage to minimize mycotoxin contamination.

Keywords: *Aspergillus*, Dried fruits and nuts, Fungi, Health risks, Mycotoxin

1. Introduction

Fungi are common microorganisms in the environment that can colonize a wide range of food sources under favorable conditions, including sufficient moisture, oxygen, and nutrients. This colonization often leads to fungal spoilage, which is evident through visible changes in food appearance, such as mold growth, as well as off-flavors, odors, and alterations in texture (Pitt and Hocking, 2009). Fungal degradation causes significant economic losses, particularly for exporting countries (Drusch and Ragab, 2003). Nuts and dried fruits are among the food categories that are most susceptible to fungal spoilage. Despite being valued for their nutritional benefits, extended shelf life, and convenience, these foods are particularly vulnerable to fungal contamination during the various stages of production, storage, and distribution (Kader and Hussein Awad M, 2009). Although the drying process reduces the moisture content in nuts and dried fruits, the residual moisture can still support fungal growth under certain conditions (González-Curbelo and Kabak, 2023). Moreover, fluctuations in environmental conditions like humidity and temperature during transportation and storage can create favorable environments for fungal proliferation in these food items (Preusser et al., 2019).

Fungi, particularly species of the genera *Aspergillus*, *Penicillium*, and *Fusarium,* are commonly associated with contamination in these foods (Pitt and Hocking, 2009). Their enzymatic activity can degrade the food matrix, leading to undesirable changes in texture, flavor and nutritional value (Dalié et al., 2010). Such contamination raises major concerns for both producers and consumers due to its negative impact on product quality and safety. These fungi pose serious health risks through the production of mycotoxins, which are toxic secondary metabolites. Mycotoxins are well known for their carcinogenic, immunosuppressive, and other toxic effects, posing serious threats to human health (Pitt and Hocking, 2009). Therefore, fungal contamination represents a significant food safety and public health concern, particularly in tropical regions like Bangladesh, where warm and humid conditions favor fungal growth and mycotoxin production.

In Bangladesh, dried fruits and nuts are widely consumed as snacks, in desserts, and during special occasions, often imported from countries where post-harvest handling and storage conditions may be suboptimal. Moreover, locally produced nuts such as peanuts are frequently stored in ambient conditions that are conducive to fungal proliferation. Studies conducted in neighboring countries have reported high levels of fungal contamination and mycotoxins in similar products (Abbas et al., 2019; Jogee et al., 2012). Despite the growing consumption of dried fruits and nuts in Bangladesh, limited studies have systematically screened these products for fungal contamination, creating a critical gap in the literature and highlighting the urgent need for focused research. Although several studies have investigated fungal and mycotoxin contamination in staple crops like rice, maize, and wheat in Bangladesh (Akter et al., 2025; Roy et al., 2013), few have focused on dried fruits and nuts. However, there is a lack of comprehensive data on the prevalence and types of fungal contaminants in dried fruits and nuts in Bangladesh, highlighting a significant research gap in this domain. This gap is particularly concerning in light of the growing consumption of these food products and their potential to harbor harmful fungi and mycotoxins.

Furthermore, most existing studies rely on traditional culture-based methods for fungal detection, which are time-consuming and may underestimate fungal diversity. This study aims to systematically assess fungal contamination in dried fruits and nuts available in the Bangladeshi market using both traditional and molecular detection methods. The findings will provide critical insights into the prevalence and diversity of fungal contaminants. By addressing the existing research gaps, this study aims to provide valuable data that can inform food safety regulations, public health interventions, and best practices for handling and storage.

2. Materials and Methods

2.1 Sampling

To assess fungal contamination, widely consumed dates, nuts, and raisins were collected from various local markets across Dhaka city during the study period. The sampling strategy was designed to include all available varieties of these products to capture the diversity of items accessible to consumers. Samples were stored in sterile zipper bags at 4 °C until analysis, and 100 g of each sample were used for fungal isolation.

2.2 Chemicals and Reagents

Potato Dextrose Agar (PDA) was purchased from HiMedia, India. Polymerase chain reaction (PCR) reagents and DNA ladder were purchased from Fisher Scientific. Primers were purchased from Integrated DNA Technologies (IDT). Agarose gels were obtained from Bio-Rad.

2.3 Moisture Content of the Samples

After drying the samples for five hours at 105 ºC, their moisture content was determined using Equation 1.

$$\text{Moisture content} = \frac{W1-W2}{W1} \times 100 \qquad (1)$$

W_1: Sample weight before drying, W_2: Sample weight after drying

2.4. Isolation of Fungi from the Samples

2.4.1 Direct Plating

The dried fruits and nuts were disinfected externally by soaking them in a 2% sodium hypochlorite solution for 1 minute, then rinsing them with sterile water and gently blotting them with tissue paper. The samples were subsequently prepared for fungal isolation by carefully cutting them into small pieces under aseptic conditions. Fungal isolates were obtained using the direct plating technique on potato dextrose agar (PDA) following standard procedures (Magan, 2006), with the addition of 0.03 mL of lactic acid per Petri dish to suppress bacterial growth.

2.4.2 Incubation

Following inoculation, the Petri dishes were incubated at 25 ± 2 °C for a period of 5 to 7 days. The plates were checked daily for growth and sporulation. Following incubation, the fungal colonies were counted, and the results were reported as colony-forming units per gram (CFU/g).

2.5 Identification of Isolated Fungi

After incubation, the isolates were purified on potato dextrose agar (PDA) and identified using their morphological and cultural characteristics, as well as molecular markers.

2.5.1 Macroscopic Characteristics

Initially, after incubation, the macroscopic characteristics of the fungal colonies, including colony color, the reverse side of the colony, pigmentation, texture, and surface appearance, were carefully examined, and recorded.

2.5.2 Microscopic Characteristics

For observation of microscopic characteristics, the mycelia and spores were examined under a microscope using the Lactophenol Cotton Blue (LCB) wet mounting method. In this technique, a drop of LPCB was placed on a clean, grease-free slide, and then, with the help of a sterile fungus needle, a tiny piece of fungal structures, such as mycelia, spore-bearing structures, and spores, was transferred into the lactophenol

cotton blue drop on the slide. The growth was gently teased with the help of a sterile fungus needle. A clean coverslip was placed over the specimens, and any excess fluid was removed by gently soaking them with blotting paper. Then the prepared slide was finally examined under the microscope using both low-power (10X) and high-power (40X) magnification (Abbas et al., 2019). The microphotographs of the fungi, along with the measurement of spore size, were taken by a high-resolution microscope facilitated with a camera (Nikon Optiphot-2 trinocular microscope, Japan) and identified based on morphological characteristics. The identification followed established protocols from various studies (Pitt, 1985; MA., 2002; Pitt and Hocking, 2009; Frisvad and Samson, 2004).

2.5.3 Molecular Identification of Fungi

2.5.3.1 DNA Extraction

The fungal DNA was extracted using the established literature protocols (Arafat et al., 2022) with minor modifications. To extract genomic DNA, fungi were cultured on PDA medium at 25 ± 2°C for 7 days, and then the fungal mycelium was collected by gently scraping the surface of the 7-day-old cultures using a sterile spatula. Fifty milligrams of fungal mycelium from the young culture were transferred to a 1.5 ml sterile Eppendorf tube using a sterile spatula and left at room temperature for 10 minutes. The mycelium was then pulverized with a microtube pestle in 500 µl of lysis buffer, which contained 400 mM Tris-HCl (pH 8.0), 60 mM EDTA (pH 8.0), 150 mM NaCl, and 1% SDS. Following this, 150 µl of KAC (pH 4.8), prepared from 60 ml of 5 M potassium acetate, 11.5 ml of glacial acetic acid, and 28.5 ml of distilled water, was added to the mixture. The mixture was vortexed for 2 minutes and then centrifuged at 12,000 rpm for 2 minutes. Then the supernatant was transferred to a fresh Eppendorf tube and centrifuged again under the same conditions. The supernatant was then transferred to another new Eppendorf tube, and an equal volume of ice-cold isopropyl alcohol was added. The tube was mixed briefly by inversion and stored at −20°C for 1 hour. Afterward, the tube was centrifuged at 12,000 rpm for 4 minutes, and the supernatant was discarded. The DNA pellet was washed with 300 µl of 70% ethanol, followed by centrifugation at 12,000 rpm for 2 minutes, after which the supernatant was discarded. The DNA pellet was air-dried and then suspended in 30 µL of 1x TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0). Then the DNA was allowed to dissolve overnight at 4 °C and was subsequently stored at -20°C for further analysis.

2.5.3.2 Assessment of the Quantity and Quality of the Extracted DNA Sample

The purity and concentration of the obtained genomic DNA were measured using a NanoDrop spectrophotometer. Qualitative analysis of the DNA sample was performed by electrophoresis using 1.5% agarose gel before Polymerase chain reaction (PCR) amplification.

2.5.3.3 Polymerase Chain Reaction

PCR amplification was performed using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') as forward and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as reverse primers. PCR amplification was performed in 0.2 mL microcentrifuge tubes in a 10 µL reaction volume containing 1 µl of 30 to 40 ng template DNA, 3.3 µl of master mix, and 5.7 µl of nuclease-free water. The master mix was prepared by combining 1 µl of 10x PCR buffer, 0.8 µl of 25 mM $MgCl_2$, 0.6 µl of 25 mM dNTPs, 0.3 µl of each of the primers (25 pmol ITS1 and ITS4), 0.3 µl of 1.0 U Taq polymerase. Then the PCR reaction mixture was spun, and PCR amplification was carried out using a C1000 Thermal Cycler, programmed with an initial denaturation step at 94 °C for 5 minutes, 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 54 °C for 30 seconds, extension at 72 °C for 30 seconds, and final extension at 72 °C for 5 minutes (Amer et al., 2011). After the final amplification cycle, the reaction tubes were held at 4 °C.

2.5.3.4 Gel Electrophoresis

The PCR products were subjected to electrophoresis in 1.5% agarose gels to determine the band sizes and to confirm amplification of the DNA. For this, 2 µL of the amplified PCR products were electrophoresed on 1.5% agarose gel in a gel-running kit containing 1xTAE buffer for 45 minutes at 100 volts and 200 mA until the DNA fragment was separated well (Armstrong and Schulz, 2015). After completion of the gel run, the gel was stained with ethidium bromide, which was added to a final concentration of 0.5 µg/ml. Electrophoresis was performed with 3 µl of a 100 bp DNA ladder alongside the reaction sample. Then the DNA band was visualized under a UV transilluminator and photographed using a Gel Documentation system (Cleaver Scientific's MultiSUB TM).

2.5.3.5 Purification of PCR Amplified Product

The PCR product was purified using the FavorPrep™ GEL/PCR purification kit following a standardized protocol. At first, the PCR product was transferred to a 1.5 ml Eppendorf tube, and FADE buffer was added in a volume five times that of the PCR product. The mixture was thoroughly mixed and briefly spun for 3 to 4 seconds before being transferred to the FADE column, which was placed in a collection tube. The sample was then centrifuged at 11,000 rpm for 30 seconds, and the supernatant was discarded. Subsequently, 750 µl of wash buffer: 100% ethanol (1:4) was added to the column and centrifuged again under the same conditions. After discarding the supernatant, an additional centrifugation step was performed at 12,000 rpm for 4 minutes to remove residual wash buffer. The FADE column was then transferred to a fresh Eppendorf tube, and an elution buffer, equivalent in volume to the PCR product, was added. The sample was left to stand for 1 minute before being centrifuged at 12,000 rpm for 3 minutes. After a final incubation of 5 minutes, a purified DNA fragment was obtained for further analysis.

2.5.3.5 Cycle Sequencing of the Purified PCR Product

Cycle sequencing facilitates sequencing of a species (Kretz et al., 1994). Prior to cycle sequencing, the quality and quantity of the purified DNA samples were evaluated using a NanoDrop spectrophotometer. Master mixtures for cycle sequencing were prepared separately for forward and reverse primers. Each master mixture (master mix 1 and master mix 2) consisted of 1.7 µl of buffer, 0.25 µl of either the forward or reverse primer, and 0.8 µl of Big Dye, yielding a total volume of 2.75 µl per reaction. For each sample, two reaction mixtures were prepared using the respective master mixtures. Each reaction contained 1 µl of template DNA, 2.75 µl of the appropriate master mixture, and 6.25 µl of nuclease-free water, resulting in a final reaction volume of 10 µl. Cycle sequencing was performed in a thermal cycle under optimized conditions. The protocol began with an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 40 seconds, annealing at 54°C for 40 seconds, and extension at 72°C for 40 seconds. A final extension was carried out at 72°C for 5 minutes, after which the reaction was held at 4°C. The PCR tubes were then stored at -20°C for further analysis.

2.5.3.6 Purification after Cycle Sequencing

Before sequencing, the PCR product was purified following a standardized protocol. Initially, 10 µl of the PCR product was transferred into a 1.5 ml Eppendorf tube, and 12 µl of master mix 1 (containing nanopure water and 125 mM EDTA) was added. The solution was thoroughly mixed by pipetting and briefly spun. After 3–4 minutes, 52 µl of master mix 2 (comprising 3M NaOAc and 100% ethanol) was added, mixed well, and incubated at room temperature for 15 minutes. The sample was then centrifuged at 13,300 rpm for 20 minutes, and the supernatant was discarded. To further purify the product, 250 µl of 70% ethanol was added, followed by another centrifugation at 13,300 rpm for 15 minutes. The supernatant was discarded, and the tube was left to air dry. Subsequently, 12–15 µl of Hi-Di Formamide was added, and the sample was incubated in the dark

for 15 minutes. Finally, the purified sample was transferred to a PCR tube, denatured at 95°C for 3 minutes, and immediately chilled on ice before further processing.

2.5.3.7 Sequencing and Species Identification

After purification, the DNA sample was sequenced through an automated sequencer. After sequencing, the resulting ITS-rDNA sequences were compared with the other sequences available in the NCBI GenBank database using the basic local alignment search tool (BLAST) algorithm (www.ncbi.nlm.nih. gov/BLAST). A match with ≥ 99% similarity was considered an identification of the fungal species.

2.6 Data Analysis

The isolation frequency of fungal species from samples was calculated using Equation 2 and the percentage of occurrence of fungal isolates was calculated using Equation 3 (Saadullah and Abdullah, 2015):

$$\text{Isolation Frequency \%} = \frac{\text{Number of samples on which a fungus appeared}}{\text{Total number of tested samples}} \times 100 \quad (2)$$

$$\text{\% of Occurrence of Isolated Fungi} = \frac{\text{Total number of colonies of the same fungi}}{\text{Total number of colonies of different fungi}} \times 100 \quad (3)$$

2.7 Statistical Analysis

All analyses were conducted in triplicate. Mean values and standard deviations were calculated using SPSS version 26. The association between the presence of *Aspergillus flavus* and the presence of aflatoxin was also evaluated using SPSS version 26.

2.8 Evolutionary Tree Construction

To explore genetic relatedness, the evolutionary tree of the obtained fungal sequences of the internal transcribed spacer region was generated by using MEGA11 using the neighbor-joining algorithm with bootstrap values (n = 1000 replicates).

3. Results

3.1 Moisture Content of the Collected Samples

The mean moisture content was highest in dates (11.42 ± 0.52%), followed by raisins (9.92 ± 0.64%) and nuts (7.98 ± 0.46%) (Table 1).

Table 1. Moisture content of collected dried fruits and nuts

Sample name	% of moisture content (mean ± SD)
Dates	11.42 ± 0.52
Raisins	9.92 ± 0.64
Nuts	7.98 ± 0.46

Table 2 presents fungal contamination levels in various nuts and dried fruits, measured in colony-forming units per gram (CFU/g). Among the analyzed samples, nuts exhibited the highest fungal contamination, with a mean of 84.5 ± 3.20 CFU/g, followed by raisins (72.8 ± 3.81 CFU/g) and dates (58.0 ± 4.24 CFU/g).

Table 2. Fungal contamination levels in various tree nuts and dried fruits

Food category	Mean ± SD
Dates	58.0 ± 4.24
Raisins	72.8 ± 3.81
Nuts	84.5 ± 3.20

The isolation frequencies of the isolated fungal species from dates, raisins, and nuts are shown in Figure 1. *Aspergillus* species were the most frequently isolated fungi, with *A. niger* and *A. flavus* detected in 95.23% and 80.95% of the samples, respectively. Among *Penicillium* species, *P. oxalicum* was the most prevalent, detected in almost all categories with an isolation frequency of 85.71%, whereas *P. chrysogenum* and *P. rubens* were less common. *Rhizopus* species were present in all food categories, particularly in raisins and nuts, with an isolation frequency of 47.61%. *Fusarium* species were detected only in dates, with a lower isolation frequency of 23.80%. These findings indicate that nuts and dried fruits harbor a diverse range of fungal contaminants, with *Aspergillus* and *Penicillium* being the dominant genera, potentially posing a risk for mycotoxin contamination.

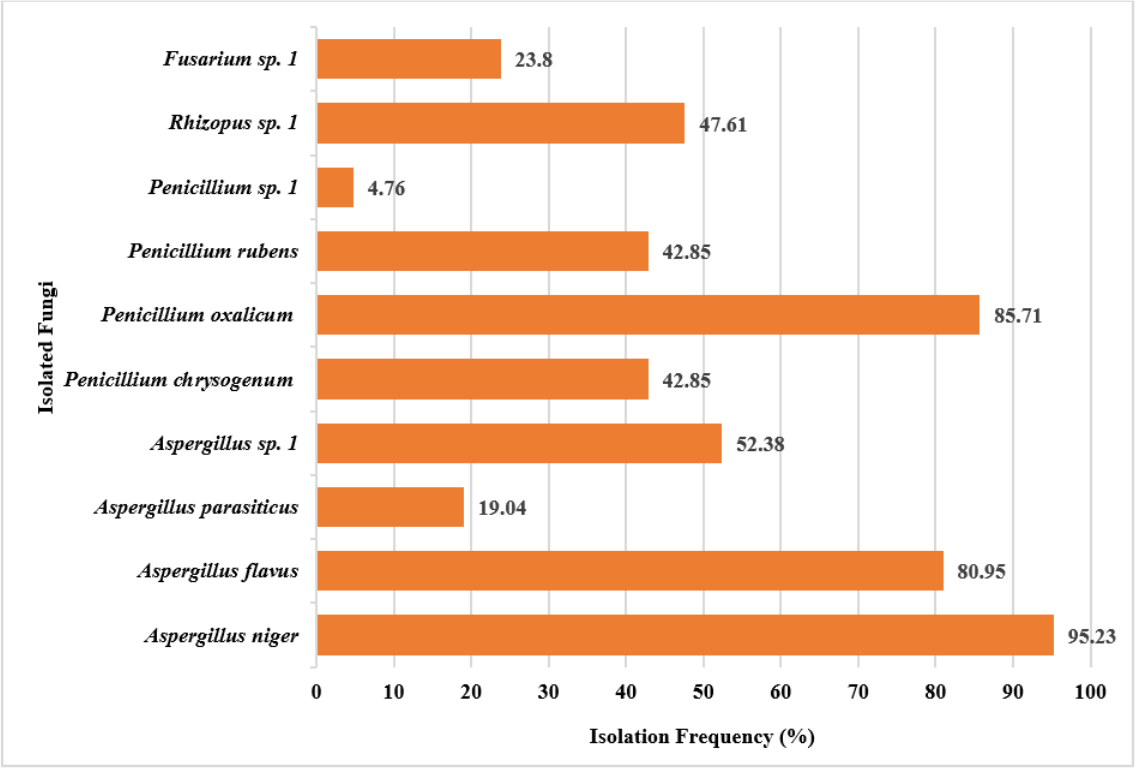


Figure 1. Isolation frequency of the fungal species isolated from dried fruits and nuts

The percentages of the occurrence of fungal species isolated from dried fruits and nuts are shown in Figure 2. The results indicate that *A. niger* was the most frequently isolated fungal species from dried fruits and nuts, accounting for 43.97% of all isolates. Other notable species included *P. oxalicum* (13.8%) and *A. flavus* (10.41%), suggesting a predominance of *Aspergillus* and *Penicillium* genera, indicating a high prevalence of potentially mycotoxigenic fungi.

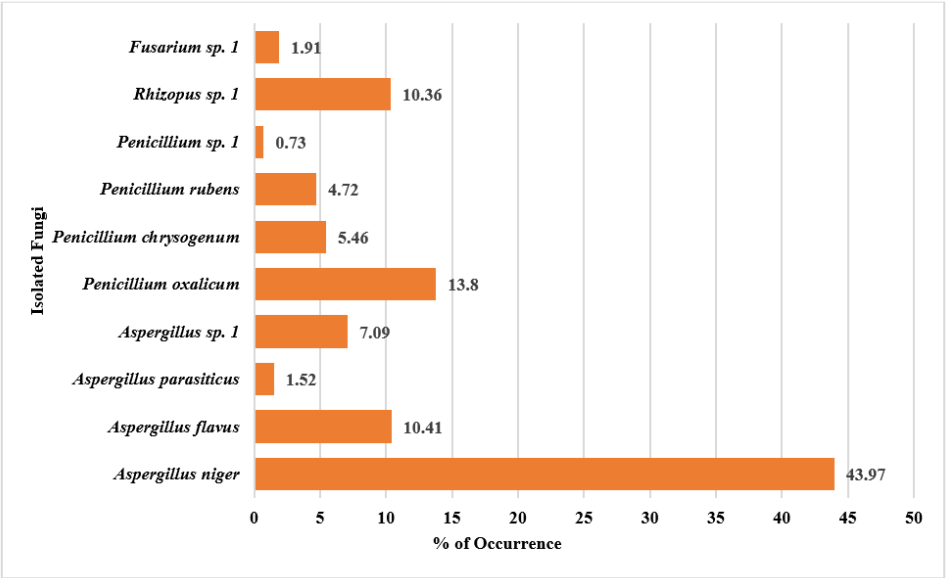
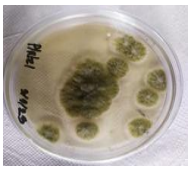
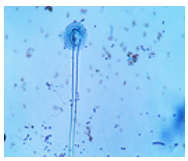
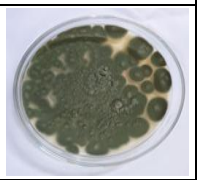
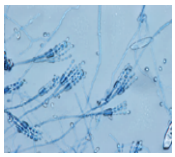
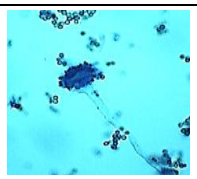
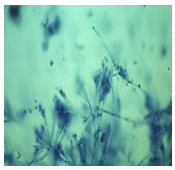
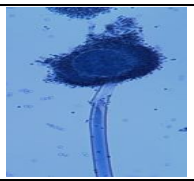
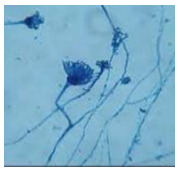
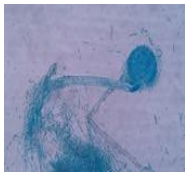
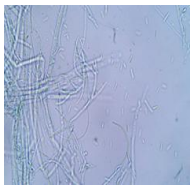


Figure 2. Percentage of the occurrence of the fungal species isolated from dried fruits and nuts

Table 3 presents representative images obtained from both macroscopic and microscopic examinations of the samples using the Lactophenol Cotton Blue (LCB) wet mount technique.

Table 3. Images of macroscopic and microscopic observation using wet mount technique

Isolated fungi	Macroscopic image	Microscopic image	Isolated fungi	Macroscopic image	Microscopic image
A. flavus			*P. oxalicum*		
A. parasiticus			*P. chrysogenum*		
A. niger			*P. rubens*		
Aspergillus sp. 1			*Penicillum sp.* 1		
Rhizopus sp. 1			*Fusarium sp.* 1		

The results of the LCB wet mount technique of various fungal species isolated from the samples are shown in Table 4. *Aspergillus* species (*A. flavus, A. parasiticus,* and *A. niger*) exhibited distinctive characteristics, with *A. flavus* forming yellowish-green colonies with blue spores and *A. parasiticus* showing dark green colonies with bluish to greenish, cottony mycelium. *Penicillium* species (*P. oxalicum, P. chrysogenum,* and *P. rubens*) displayed varying colony colors, from green to blue-green, and had brush-like or unbranched conidiophores with blue spores. *Rhizopus* sp. formed fluffy white growths with brown spores and rhizoids, while *Fusarium* sp. initially presented white colonies that turned yellow or orange, with boat-shaped macroconidia and single-celled microconidia. These observations highlight the diversity of fungal characteristics, which are essential for their identification and potential risk assessment in food products.

Table 4. Colony morphology of fungi on PDA plates and microscopic observation and microscopic observation using wet mount technique

Isolated fungi	Colony morphology		Microscopic observation		
	Upper surface	Reverse surface	Mycelium	Spores	Conidiophores/Sterigmata
A. flavus	Yellowish green	Reddish-gold	Blue mycelia, woolly texture	Blue spores	Conidiophores are asexual spores, rough, colorless, and variable in length with spherical to sub-spheroidal vesicles
A. parasiticus	dark green colonies	yellow-to-orange	White to bluish to greenish, cottony or velvety texture	Blue spores, round to oval in chains	Conidiophores are variable in length, with spherical vesicle, uniseriate conidial head, and short and spiny sterigmata
A. niger	White to dark brown then turn to black	yellowish white	Black/brown mycelium, septate and well-developed	Black spores	Conidiophores are variable in length with radiating and biseriate conidial heads and globose vesicles
Aspergillus sp. 1	Grayish-white	Yellowish -white	White to bluish mycelium, septate, and branched	Blue spores	Conidiophores are long, smooth-walled, globose to sub-globose vesicles
P. oxalicum	Initially white, then turns to green	Pale to brownish-yellow	White to bluish-green mycelium, cottony texture, and radiating outward	Blue spores	Conidiophores are branched, appear like a brush, narrow and smooth-walled
P. chrysogenum	Greenish to blueish-green,	Pale to yellowish	White to bluish-green mycelia, powdery, or woolly	Bluish-green spores	Conidiophores are branched, smooth-walled, forming brush-like clusters
P. rubens	Blue or bluish-green tint	Yellow to light brown	Light blue mycelium, cottony texture	Blue spores	Conidiophores are unbranched, smooth, variable in length with globose vesicle
Penicillum sp. 1	white, floccose	Pale	White to blue mycelium	Blue spores	Long, variable in length, terverticillate conidial heads
Rhizopus sp. 1	White fluffy, grayish brown with black spores	Pale	Brown mycelium with branched hyphae, which can be divided into stolons, rhizoids, and sporangiophores	Brown spores	Rhizoids are present
Fusarium sp. 1	Initially white, later turns yellow to orange	Yellowish to brownish	White to pinkish or cream-colored mycelium, branched, ranging from sparse to profuse, and floccose	Macroconidia : elongated, boat-shaped spores; Microconidia - oval, single-celled spores	Conidiophores are variable in length, often smooth, and unbranched, conidia emerging from the tips of the sterigmata in chains

PCR amplification with ITS1 and ITS4 primers produced distinct bands of the expected size (~ 560 bp) between the 500 bp and 600 bp markers of the 100 bp DNA ladder for all DNA samples, confirming successful

amplification (Figure 3). Subsequent sequence analysis of the ITS region verified the identity of *Aspergillus flavus*, *A. parasiticus*, *A. niger*, *Penicillium oxalicum*, *P. chrysogenum*, and *P. rubens*, each showing 100% similarity with corresponding reference sequences in the NCBI database. These molecular results corroborated the morphological identification of the isolates.

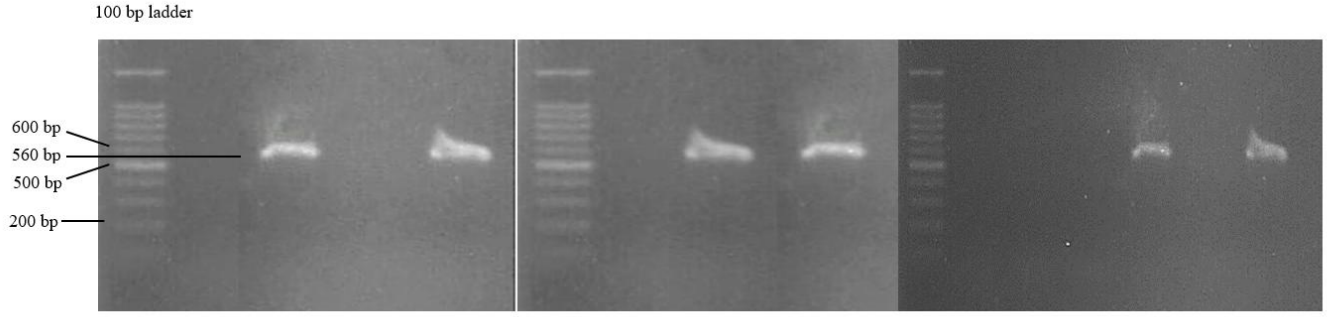


Figure 3: Images of gel electrophoresis of PCR-amplified DNA samples of the isolated fungal species

The phylogenetic analysis based on ITS sequences revealed clear clustering of *Aspergillus* and *Penicillium* isolates obtained from dates, raisins, and nuts with their corresponding reference strains from the NCBI database. The neighbor-joining algorithm in MEGA11, using *Neurospora crassa* as an outgroup, showed strong bootstrap support (n = 1000), confirming the robustness of the branches. Isolates from the present study grouped consistently within their respective species clades and are indicated in blue, with accession numbers provided as unique identifiers (Figure 4).

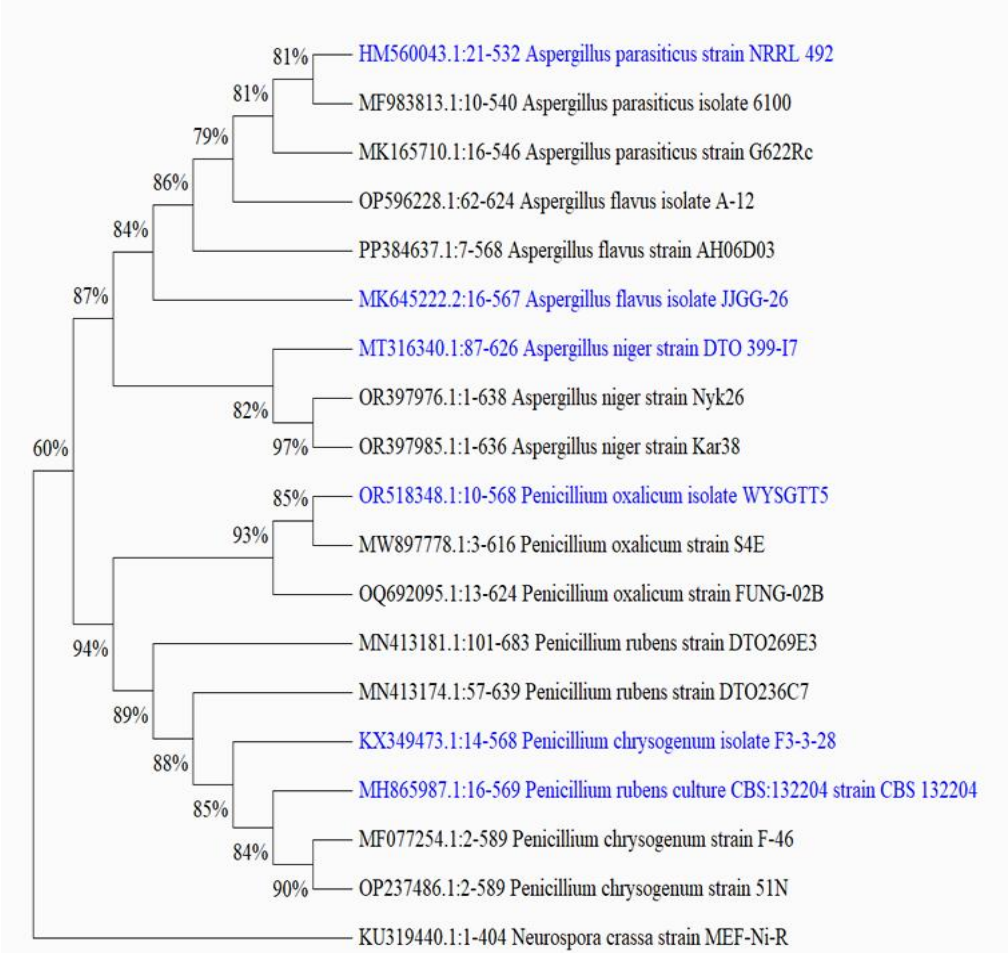


Figure 4. Phylogenetic tree of internal transcribed spacer region sequences of isolated *Aspergillus* and *Penicillium* species with related NCBI reference sequences

4. Discussion

Dried fruits and nuts are highly susceptible to contamination by mycotoxin-producing fungi (Kishore et al., 2002). The study assessed the prevalence of fungal contamination levels in dried fruits and nuts. The mean fungal colony counts were 84.5 ± 3.20 CFU/g for nuts, 72.8 ± 3.81 CFU/g for raisins, and 58.0 ± 4.24 CFU/g for dates, indicating that nuts harbored the highest fungal load while dates had the lowest. These findings are in agreement with earlier studies reporting a higher fungal load in nuts compared to dried fruits (Tournas et al., 2015). These findings support previous studies indicating that nuts are particularly vulnerable to fungal colonization due to their nutrient-rich composition and extended shelf life (Adetunji et al., 2021).

In this study, a total of 10 different fungal species belonging to 4 genera, *Aspergillus, Penicillium, Rhizopus,* and *Fusarium*, were isolated from the dried fruit and nut samples. In comparison, a previous study reported 38 fungal species belonging to 13 genera in dried fruit samples from Duhok (Saadullah and Abdullah, 2015), whereas another study identified 28 species in dried fruits and nuts from India (Jogee et al., 2012). The discrepancies observed between our results and those of the studies may be attributed to the inherent diversity in fungal flora influenced by local environmental conditions and methodological differences. Initial identification of the fungal isolates was based on morphological and microscopic characteristics. Subsequently, the presence of six fungal species—*A. flavus*, *A. parasiticus*, *A. niger, P. oxalicum, P. chrysogenum,* and *P. rubens*—was later confirmed through ITS-rDNA sequencing. A similar molecular approach for fungal species identification was employed in a previous study (Jogee et al., 2012). The ITS region, widely regarded as the universal DNA barcode for fungi, has been shown to possess sufficient interspecific variability for reliable species-level identification in filamentous fungi, including *Aspergillus* and *Penicillium* (Schoch et al., 2012).

In our study, *Aspergillus* was the most dominant fungal genus, appearing in 95.23% of the samples and accounting for 43.97% of the total isolates. This dominance may be attributed to the ability of *Aspergillus* species to thrive in low-moisture conditions and their widespread occurrence in soil and organic matter, which facilitates contamination during processing and storage (de Vries et al., 2017). These findings are consistent with previous studies (Magan et al., 2019; Tournas et al., 2015). The most frequently detected fungi were *A. niger, A. flavus, P. oxalicum,* and *Rhizopus*, aligning with findings from previous studies (Khlangwiset and Wu, 2011). Notably, *A. niger* was present in almost all samples, which agrees with reports from Argentina and Brazil (Iamanaka et al., 2005; Romero et al., 2005). The high prevalence of *A. niger* is expected, as its black spores are relatively resistant to UV radiation and sunlight, allowing it to survive the drying process (Romero et al., 2005). *A. flavus* was detected in approximately four out of five samples, while *A. parasiticus* was present in only about one in five samples. This substantial difference in prevalence is consistent with previous research indicating that *A. flavus* is more commonly found in food products than *A. parasiticus* (Luttfullah and Hussain, 2011). Both species are recognized producers of aflatoxins; however, *A. flavus* exhibits broader distribution due to its adaptability and ubiquitous nature (Chang et al., 2014). Consequently, *A. flavus* has been isolated from a wide variety of food items (Chang et al., 2014).

In this study, the evolutionary relationships among the identified *Aspergillus* and *Penicillium* species were elucidated based on the ITS region. The use of the neighbor-joining algorithm in MEGA11, along with 1000 bootstrap replicates, provided a robust methodology for assessing the genetic relatedness of the isolates. The resulting clustering patterns in the evolutionary tree were consistent with existing taxonomic classifications, with the isolates aligning closely with their respective reference strains retrieved from the NCBI GenBank database. This congruence highlights the reliability of ITS-based phylogenetic approaches in resolving taxonomic identities within these fungal genera (Schoch et al., 2012). Furthermore, the inclusion of *Neurospora crassa* as an outgroup allowed for accurate rooting of the tree and ensured that evolutionary

inferences were drawn with appropriate context (Brown, 2002). Bootstrap support values at major nodes, typically exceeding 70%, further affirmed the reliability of the inferred evolutionary relationships, in line with widely accepted thresholds for phylogenetic confidence (Simpson, 2010). These values indicate that the evolutionary relationships depicted are not due to random chance but rather reflect consistent patterns in the data across replicates.

This study offers valuable insights into fungal contamination in dried fruits and nuts in Bangladesh, addressing a significant gap in the existing literature. The reliability of the findings was strengthened by the use of both traditional methods and molecular techniques, including ITS-rDNA sequencing and phylogenetic analysis, which enabled accurate identification of fungal species. Additionally, the use of an efficient and safe DNA extraction protocol further contributed to the reliability of the results. However, to enhance generalizability and obtain a more representative national overview, future studies should incorporate a larger, geographically diverse sampling framework. Furthermore, longitudinal studies with year-round sampling are recommended to assess seasonal variations in fungal contamination and offer a more comprehensive understanding of the fungal ecology associated with dried fruits and nuts in Bangladesh.

5. Conclusion

This study provides the first comprehensive assessment of toxigenic fungi in dried fruits and nuts available in local markets across Bangladesh. The findings highlight the dominance of *Aspergillus* and *Penicillium* species, particularly *A. niger* and *A. flavus*, underscoring the significant risk of mycotoxin contamination in these commonly consumed food products. The use of both morphological and molecular identification methods, including ITS-based sequencing, ensured accurate characterization of fungal species. While the findings offer important baseline data, future studies should incorporate larger sample sizes, broader geographic coverage, and year-round sampling to capture seasonal variations and improve the generalizability of results. Strengthening food safety practices, coupled with regular monitoring, is essential to mitigate public health risks associated with mycotoxins in dried fruits and nuts.

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Data Availability Statement: *The data supporting the findings of this study, including fungal isolation records, colony count data, and species identification results, are available from the corresponding author upon reasonable request.*

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